Data Evaluation Record on the Acute Toxicity of Glufosinate-ammonium Technical to Algae, *Anabaena flos-aquae*

EPA MRID Number 48444816

Data Requirement:	EPA DP Barcode	345709
	EPA MRID	48444816
	EPA Guideline	850.5400

Test material: **Glufosinate-Ammonium Technical** **Purity:** 99.2%
Common name
Chemical name: IUPAC Ammonium; 4-[hydroxyl(methyl)phosphinoyl])-DL-homoalanine
CAS name 2-amino-4-(hydroxymethylphosphinyl)butanoic acid, monoammonium salt
CAS No. 77182-82-2
Synonyms

Primary Reviewer: Moncie Wright **Signature:** Moncie V Wright
Staff Scientist, Cambridge Environmental Inc. **Date:** 7/27/11

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental Inc. **Date:** 10/20/11

Primary Reviewer: Catherine Aubee **Signature:**
Biologist, US EPA/OPP/EFED/ERBIV **Date:** 1 June 2012

EPA PC Code 128850

Date Evaluation Completed: 01-06-2012

CITATION: Banman, C.S., J.H. Howerton, and C.V. Lam. 2011. Toxicity of Glufosinate-ammonium to the Blue Green Algae *Anabaena flos-aquae*. Unpublished study performed and sponsored by Bayer CropScience (laboratory located in Stilwell, Kansas and sponsor located in Research Triangle Park, North Carolina). Study completed March 25, 2011.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Anabaena flos-aquae* were exposed to **Glufosinate-Ammonium Technical** at nominal concentrations of 0 (negative control), 3.8, 12, 39, 125, and 400 µg ai/L under static conditions. Mean-measured concentrations were <LOQ (<1.0, control), 4.0, 13, 41, 128, and 421 µg ai/L.

The most sensitive endpoint was cell density, with NOAEC and EC_{50} values of 41 and 72 µg ai/L, respectively. The % growth inhibition of cell density in the treated algal culture as compared to the control ranged from -23 to 99%.

No abnormal observations of cell morphology were noted.

This toxicity study is scientifically sound is classified as **acceptable**. It satisfies the guideline requirement for a Tier II algal toxicity study using glufosinate technical.

Results Synopsis

Test Organism: *Anabaena flos-aquae*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}: 30 µg ai/L 95% C.I.: 10-88 µg ai/L
EC_{50}: 72 µg ai/L 95% C.I.: 44-120 µg ai/L
NOAEC: 41 µg ai/L
Probit Slope: 4.29 ± 1.51

Biomass
EC_{05}: 36 µg ai/L 95% C.I.: 19-69µg ai/L
EC_{50}: 81 µg ai/L 95% C.I.: 62-110 µg ai/L
NOAEC: 41 µg ai/L
Probit Slope: 4.68 ± 1.10

Growth rate
EC_{05}: 47 µg ai/L 95% C.I.: 26-85 µg ai/L
EC_{50}: 150 µg ai/L 95% C.I.: 110-190 µg ai/L
NOAEC: 41 µg ai/L
Probit Slope: 3.34 ± 0.531

Endpoint(s) Effected: Cell density, biomass, and growth rate

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test procedure followed the guidelines of the Organization for Economic Cooperation and Development (OECD) Guideline No. 201; OPPTS Guideline 850.5400; and FIFRA Guideline 123-2. The study methods and results were evaluated according to U.S. EPA OPPTS 850.5400: Algal Toxicity, Tiers I and II and OECD No. 201 Alga, Growth Inhibition Test, and differences and/or similarities were described. One deficiency and deviations from OPPTS 850.5400 and OECD 201 were noted:

1. The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

2. The physico-chemical properties of the test material were not reported; OECD guidelines suggest that this information be reported. OPPTS guidelines do not address this topic.

3. The pH of the control ranged from 7.4 to 9.0 and in the test solutions ranged from 7.4 to 9.4; OPPTS guidelines suggest a pH of 7.5 ± 0.2 for similar algal species. Additionally, OECD guidelines suggest that the control pH not vary by more than 1.5 units.

4. The strain of the algal species was not reported.

5. The source of the dilution water was not reported.

The deficiency and deviations do not substantively impact on the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the Good Laboratory Practice Standards as specified in 40 CFR Part 160.

A. MATERIALS:

1. Test material **Glufosinate-Ammonium Technical**

Description: White solid

Lot No./Batch No. : 1207200501 (Batch ID)

Purity: 99.2%

Stability of compound under test conditions: Analytical verification at time 0 yielded recoveries ranging from 103 to 113% of the nominal test concentrations. Recoveries from the day 4 solutions ranged from 101 to 111% of nominal. The test material was very

stable under the test conditions.
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored in a freezer.

Physicochemical properties of Glufosinate-Ammonium Technical.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Blue-green algae; *Anabaena flos-aquae*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: Not reported
Source: In-house cultures originally obtained from the University of Texas, Austin, Texas, United States.
Age of inoculum: 3 days
Method of cultivation: Algae were cultivated in 1xAAP nutrient medium.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A 96-hour range-finding study was conducted from January 6 to January 10, 2011 with a control and nominal concentrations of 0.16, 0.8, 4.0, 20, and 100 mg ai/L. After 96 hours, there was a significant reduced cell density in the lowest test level and virtually no growth in the higher test levels. An additional study was necessary to establish the test levels for the definitive test.
A second test was conducted from January 11 to 15, 2011 with a negative control and nominal concentrations of 0.004, 0.02, and 0.1 mg ai/L. Inhibitions in the 0.02 and 0.1 mg ai/L test levels were 10 and 32%, respectively. All cells in the test levels appeared normal as compared to the control.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Same as test (dilution water, temperature, photoperiod, and light intensity) The culture was in log phase growth.	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system Static/static renewal Renewal rate for static renewal	Static N/A	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	The test vessels were placed on an orbital shaker table in an environmental chamber.	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name		Control pH: 7.4-9.0

Parameter	Details	Remarks *Criteria*
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	7.4 7.5-9.4 Yes $NaHCO_3$ N/A	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A- standard medium was used	
<u>Dilution water</u> source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Distilled water 7.5 N/A Filter sterilized Not reported Not reported Not reported Not reported Not reported	 *EPA pH: <u>Skeletonema costatum</u>= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	The test material (201.7 mg) was dissolved in nutrient medium to create a primary stock solution, which was the highest test concentration. The solution was inverted several times, and the lower test concentrations were made via serial dilution from the next higher test concentration.	

Parameter	Details	Remarks ***Criteria***
Aeration or agitation	Agitation; 100 rpm	
Initial cells density	1 x 10^4 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates Control: Solvent control: Treatments:	 3 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations		

Parameter	Details	Remarks *Criteria*
Nominal: Measured:	0 (negative control), 3.8, 12, 39, 125, and 400 µg ai/L <LOQ (<1.0, control), 4.0, 13, 41, 128, and 421 µg ai/L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Samples from the control and all test levels were taken from the time 0 and 96 hour solutions, and were analyzed via LC/MS/MS. Laboratory spikes and calibration standards were also analyzed.	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	 23.9-24.0°C Continuous 1880-2128 lux Cool white fluorescents	 *EPA temperature: <u>Skeletonema</u>: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25ºC maintained at ± 2ºC and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
<u>Reference chemical (if used)</u> name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	- Cell density - Biomass - Growth rate	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Cell density was determined using a light microscope and hemacytometer slide. Growth rate was determined using a logarithmic equation that accounts for initial and final cell density. Biomass was determined via an equation using initial and final cell density.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	None.	
Indicate whether there was an exponential growth in the control	Yes; cell density was 154 x 10^4 cells/mL at 96 hours.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

At 96 hours, cell density in the negative control averaged 154 x 10^4 cells/mL, which yielded inhibitions of -23, 8, -2, 88, and 99% in the mean-measured 4.0, 13, 41, 128, and 421 µg ai/L test levels as compared to the control. The 96-hour NOAEC and EC_{50} values for cell density were 39 and 67 µg ai/L, respectively.

At 96 hours, biomass in the negative control averaged 3596 x 10^4 cells/mL*h, yielding inhibitions of -9, 10, 3, 83, and 100% as compared to the control. The 96-hour NOAEC and EC_{50} values for biomass were 39 and 88 µg ai/L, respectively.

At 96 hours, the growth rate in the negative control averaged 0.0523 $hours^{-1}$, yielding inhibitions of -4, 2, 0, 42, and 108%. The 96-hour NOAEC and EC_{50} values for growth rate were 39 and 129 µg ai/L, respectively.

No abnormal observations of cell morphology were noted.

Table 3: Effect of Glufosinate-ammonium Technical on algal growth of *Anabaena flos-aquae*.

Treatment **Mean-Measured (and Nominal)** **µg ai/L**	**Initial cell Density (x 10^4 cells/mL)**	**Cell density (x 10^4 cells/mL) at**			
		24 hours	**72 hours**	**96 hours**	
				cell count	**% inhibition**
Negative control	1.0	3.1	60.3	153.5	N/A
4.0 (3.8)	1.0	1.3	58.4	189.0	-23
13 (12)	1.0	3.0	54.0	140.7	8
41 (39)	1.0	1.7	56.5	155.9	-2
128 (125)	1.0	1.1	14.1	18.7	88
421 (400)	1.0	0.7	1.4	1.1	99
Reference chemical (if used)	N/A				

Table 4: Effect of Glufosinate-ammonium Technical on algal growth (*Anabaena flos-aquae*).

Treatment Mean-Measured (and Nominal) µg ai/L	Initial Cell Density ($x10^4$ cells/mL)	Mean Growth Rate (hours^{-1})		Mean Biomass ($x\ 10^4$ cells/mL*h)	
		0-96 Hours	Percent Inhibition	0-96 hours	Percent Inhibition
Negative control	1.0	0.0523	N/A	3596	N/A
4.0 (3.8)	1.0	0.0541	-4	3927	-9
13 (12)	1.0	0.0514	2	3222	10
41 (39)	1.0	0.0523	0	3504	3
128 (125)	1.0	0.0302	42	602	83
421 (400)	1.0	-0.0043	108	-14	100

Table 5: Statistical endpoint values.*

Statistical Endpoint	Cell density	Growth rate	Biomass
NOAEC (µg ai/L)	39	39	39
LOAEC (µg ai/L)	125	125	125
EC_{50} (µg ai/L) (95% C.I.)	67 (43-103)	129 (118-140)	88 (67-116)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A		

* Do not use this table, if the study was deemed unacceptable.

B. REPORTED STATISTICS:

Cell density, biomass, and growth rate data were first analyzed for normality and homogeneity of variance using using the Shapiro-Wilks test and Levene's test, respectively. If the assumptions of ANOVA were met, then the NOAEC was determined via ANOVA followed by Dunnett's test. If the data did not meet the assumptions of ANOVA, the ranks of the raw values were determined and ANOVA and a one-tailed Dunnett's test were performed on the obtained ranks.

The EC_{50} values were determined using non-linear regression analysis (the logistic model or the Bruce/Versteeg Cumulative Normal Model). The analysis was conducted using SAS statistical software and the nominal test concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer tested cell density, biomass, and growth rate replicate data for normality using the Chi-square and Shapiro Wilk's tests and for homogeneity of variance using Levene's test in Toxstat 3.5. All endpoint data met the assumptions of ANOVA, and were thus analyzed using Dunnett's and Williams' tests to determine the NOAEC. The ECx values (with 95% C.I.) and probit slope were determined using the probit analysis in Nuthatch.

All toxicity values were determined using the 96-hour mean-measured concentrations. Cell density values were entered into Toxstat 3.5 as an abbreviated value, representing the value x 10^4. Negative data values were reported for the highest test level for both growth rate and biomass; the reviewer treated those values as zeros for input into Nuthatch in order to generate ECx values.

Cell density

EC_{05}: 30 µg ai/L	95% C.I.: 10-88 µg ai/L
EC_{50}: 72 µg ai/L	95% C.I.: 44-120 µg ai/L
NOAEC: 41 µg ai/L	
Probit Slope: 4.29 ± 1.51	

Biomass

EC_{05}: 36 µg ai/L	95% C.I.: 19-69µg ai/L
EC_{50}: 81 µg ai/L	95% C.I.: 62-110 µg ai/L
NOAEC: 41 µg ai/L	
Probit Slope: 4.68 ± 1.10	

Growth rate

EC_{05}: 47 µg ai/L	95% C.I.: 26-85 µg ai/L
EC_{50}: 150 µg ai/L	95% C.I.: 110-190 µg ai/L
NOAEC: 41 µg ai/L	
Probit Slope: 3.34 ± 0.531	

D. STUDY DEFICIENCIES:

The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

E. REVIEWER'S COMMENTS:

The reviewer's and the study author's toxicity values were in general agreement when taking into consideration the difference between nominal and mean-measured concentrations. However, the reviewer obtained toxicity values using mean-measured concentrations, while the study author used nominal concentrations. Mean-measured concentrations are preferred; therefore, the reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The experiment was initiated February 21, 2011, and was terminated February 25, 2011.

F. CONCLUSIONS:

This toxicity study is scientifically sound is classified as **acceptable**. It satisfies the guideline requirement for a Tier II algal toxicity study using glufosinate technical. The most sensitive endpoint was cell density, with NOAEC and EC_{50} values of 41 and 72 µg ai/L, respectively.

Cell density
EC_{05}: 30 µg ai/L 95% C.I.: 10-88 µg ai/L
EC_{50}: 72 µg ai/L 95% C.I.: 44-120 µg ai/L
NOAEC: 41 µg ai/L
Probit Slope: 4.29 ± 1.51

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NOAEC: 41 µg ai/L
Probit Slope: 3.34 ± 0.531

Endpoint(s) Effected: Cell density, biomass, and growth rate

III. REFERENCES:

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater, 17th edition. Washington, D.C.

American Society for Testing and Materials (ASTM). 2007. Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae. ASTM Standard E1218. West Conshohocken, PA.

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82-020. Office of Pesticide Programs. Washington, D.C.

U.S. EPA. 1986. Standard Evaluation Procedure, Non-Target Plants: Growth and Reproduction of Aquatic Plants – Tiers 1 and 2. EPA-540/9-86-134. Office of Pesticides Programs. Washington, D.C.

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U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving waters to Freshwater Organisms. EP-821-R-02-013. Office of Water. Washington, D.C.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
Title:  Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L
File:             4816c             Transform:             NO TRANSFORMATION

                      Chi-Square Test for Normality
------------------------------------------------------------------------------

                     Actual and Expected Frequencies

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
             -----     -------------     -----------    -----------    -----
EXPECTED    1.2060        4.3560            6.8760         4.3560     1.2060
OBSERVED     0             7                 5              6          0

------------------------------------------------------------------------------

     Chi-Square =   5.1491             (p-value = 0.2723)

     Critical Chi-Square   = 13.277  (alpha = 0.01 , df = 4)
                           =  9.488  (alpha = 0.05 , df = 4)

------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L
File:             4816c             Transform:             NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
------------------------------------------------------------------------------

     D = 17043.3130
     W =   0.9531

     Critical W  = 0.8580 (alpha = 0.01 , N = 18)
              W  = 0.8970 (alpha = 0.05 , N = 18)

------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L
File:             4816c             Transform:             NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
------------------------------------------------------------------------------

   SOURCE             DF              SS                MS           F
------------------------------------------------------------------------------
   Between             5           4273.1656         854.6331       1.5059

   Within (Error)     12           6810.3877         567.5323
------------------------------------------------------------------------------
```

```
   Total              17          11083.5532
-----------------------------------------------------------------------------
                                                          (p-value = 0.2594)

   Critical F  = 5.0643  (alpha = 0.01, df = 5,12)
               = 3.1059  (alpha = 0.05, df = 5,12)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)


Title:  Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L
File:             4816c             Transform:             NO TRANSFORMATION

                              ANOVA Table
-----------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
-----------------------------------------------------------------------------
   Between             5          94090.9238       18818.1848      13.2497

   Within (Error)     12          17043.3130        1420.2761
-----------------------------------------------------------------------------
   Total              17         111134.2368
-----------------------------------------------------------------------------
                                                          (p-value = 0.0002)

   Critical F  = 5.0643  (alpha = 0.01, df = 5,12)
               = 3.1059  (alpha = 0.05, df = 5,12)

   Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)


Title:  Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L
File:             4816c             Transform:             NO TRANSFORMATION

      Dunnett's Test   -   TABLE 1 OF 2             Ho:Control<Treatment
-----------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN          SIG
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  0.05
----- --------------------    -----------    ------------------  ------  ---
  1            Neg control    153.5000           153.5000
  2                    4.0    189.0000           189.0000       -1.1537
  3                     13    140.6667           140.6667        0.4171
  4                     41    155.9167           155.9167       -0.0785
  5                    128     18.7400            18.7400        4.3795  *
  6                    421      1.1267             1.1267        4.9519  *
-----------------------------------------------------------------------------
Dunnett critical value = 2.5000   (1 Tailed, alpha = 0.05, df = 5,12)


Title:  Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L
File:             4816c             Transform:             NO TRANSFORMATION

      Dunnett's Test   -   TABLE 2 OF 2             Ho:Control<Treatment
-----------------------------------------------------------------------------
                           NUM OF     MIN SIG DIFF     % OF     DIFFERENCE
GROUP    IDENTIFICATION     REPS     (IN ORIG. UNITS)  CONTROL  FROM CONTROL
----- --------------------  -------  ----------------  -------  ------------
```

1	Neg control	3			
2	4.0	3	76.9273	50.1	-35.5000
3	13	3	76.9273	50.1	12.8333
4	41	3	76.9273	50.1	-2.4167
5	128	3	76.9273	50.1	134.7600
6	421	3	76.9273	50.1	152.3733

Title: Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L
File: 4816c Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	153.5000	153.5000	171.2500
2	4.0	3	189.0000	189.0000	171.2500
3	13	3	140.6667	140.6667	148.2917
4	41	3	155.9167	155.9167	148.2917
5	128	3	18.7400	18.7400	18.7400
6	421	3	1.1267	1.1267	1.1267

Title: Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L
File: 4816c Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	153.5000				
4.0	171.2500	-0.5768		1.7800	k= 1, v=12
13	148.2917	0.1693		1.8700	k= 2, v=12
41	148.2917	0.1693		1.9000	k= 3, v=12
128	18.7400	4.3795	*	1.9200	k= 4, v=12
421	1.1267	4.9519	*	1.9300	k= 5, v=12

s = 37.6866

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	30.	10.	88.	0.22	0.34
EC10	36.	14.	93.	0.19	0.39
EC25	50.	25.	1.0E+02	0.15	0.49
EC50	72.	44.	1.2E+02	0.099	0.61

Slope = 4.29 Std.Err. = 1.51

Goodness of fit: p = 0.19 based on DF= 3.0 12.

4816C : Glufosinate-ammonium & A. flos-aquae 96-hr cells; ug/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	154.	165.	-11.3	100.	0.00
4.00	3.00	189.	165.	24.2	100.	3.53e-06
13.0	3.00	141.	165.	-24.0	99.9	0.0699
41.0	3.00	156.	141.	15.1	85.5	14.5
128.	3.00	18.7	23.7	-4.93	14.4	85.6
421.	3.00	1.13	0.0852	1.04	0.0517	99.9

Title: Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L
File: 4816b2 Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.2060	4.3560	6.8760	4.3560	1.2060
OBSERVED	0	7	5	6	0

Chi-Square = 5.1491 (p-value = 0.2723)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L
File: 4816b2 Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 1574162.0000
W = 0.9741

Critical W = 0.8580 (alpha = 0.01 , N = 18)
W = 0.8970 (alpha = 0.05 , N = 18)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L
File: 4816b2 Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	330738.9444	66147.7889	0.8116
Within (Error)	12	978063.3333	81505.2778	
Total	17	1308802.2778		

(p-value = 0.5635)

Critical F = 5.0643 (alpha = 0.01, df = 5,12)
= 3.1059 (alpha = 0.05, df = 5,12)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L
File: 4816b Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	44052793.3333	8810558.6667	67.0644
Within (Error)	12	1576494.6667	131374.5556	
Total	17	45629288.0000		

(p-value = 0.0000)

Critical F = 5.0643 (alpha = 0.01, df = 5,12)
= 3.1059 (alpha = 0.05, df = 5,12)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L
File: 4816b Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Neg control	3596.0000	3596.0000		
2	4.0	3926.6667	3926.6667	-1.1173	

3	13	3222.3333	3222.3333	1.2626	
4	41	3504.6667	3504.6667	0.3086	
5	128	602.0000	602.0000	10.1168	*
6	421	-13.6667	-13.6667	12.1971	*

Dunnett critical value = 2.5000 (1 Tailed, alpha = 0.05, df = 5,12)

Title: Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L
File: 4816b Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	4.0	3	739.8608	20.6	-330.6667
3	13	3	739.8608	20.6	373.6667
4	41	3	739.8608	20.6	91.3333
5	128	3	739.8608	20.6	2994.0000
6	421	3	739.8608	20.6	3609.6667

Title: Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L
File: 4816b Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	3596.0000	3596.0000	3761.3333
2	4.0	3	3926.6667	3926.6667	3761.3333
3	13	3	3222.3333	3222.3333	3363.5000
4	41	3	3504.6667	3504.6667	3363.5000
5	128	3	602.0000	602.0000	602.0000
6	421	3	-13.6667	-13.6667	-13.6667

Title: Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L
File: 4816b Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	3596.0000				
4.0	3761.3333	-0.5587		1.7800	k= 1, v=12
13	3363.5000	0.7856		1.8700	k= 2, v=12
41	3363.5000	0.7856		1.9000	k= 3, v=12
128	602.0000	10.1168	*	1.9200	k= 4, v=12
421	-13.6667	12.1971	*	1.9300	k= 5, v=12

s = 362.4563

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	36.	19.	69.	0.13	0.53
EC10	43.	25.	75.	0.11	0.57
EC25	58.	38.	88.	0.085	0.66
EC50	81.	62.	1.1E+02	0.054	0.77

Slope = 4.68 Std.Err. = 1.10

Goodness of fit: p = 0.23 based on DF= 3.0 12.

4816B2 : Glufosinate-ammonium & A. flos-aquae 96-hr biomass;ug/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	3.60e+03	3.63e+03	-33.7	100.	0.00
4.00	3.00	3.93e+03	3.63e+03	297.	100.	4.96e-08
13.0	3.00	3.22e+03	3.63e+03	-407.	100.	0.0101
41.0	3.00	3.50e+03	3.33e+03	177.	91.7	8.31
128.	3.00	602.	642.	-39.6	17.7	82.3
421.	3.00	9.00	1.49	7.51	0.0410	100.

Title: Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L
File: 4816g Transform: NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.2060	4.3560	6.8760	4.3560	1.2060
OBSERVED	0	8	4	6	0

Chi-Square = 7.2838 (p-value = 0.1216)

Critical Chi-Square = 13.277 (alpha = 0.01 , df = 4)
= 9.488 (alpha = 0.05 , df = 4)

Data PASS normality test (alpha = 0.01). Continue analysis.

```
Title:  Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L
File:             4816g            Transform:             NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
--------------------------------------------------------------------------------

     D =   0.0002
     W =   0.9526

     Critical W  = 0.8580 (alpha = 0.01 , N = 18)
              W  = 0.8970 (alpha = 0.05 , N = 18)

--------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.



Title:  Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L
File:             4816g            Transform:             NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                              ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
--------------------------------------------------------------------------------
   Between             5              0.0000           0.0000       0.2168

   Within (Error)     12              0.0001           0.0000
--------------------------------------------------------------------------------
   Total              17              0.0001
--------------------------------------------------------------------------------
                                                          (p-value = 0.9486)

   Critical F  = 5.0643  (alpha = 0.01, df = 5,12)
               = 3.1059  (alpha = 0.05, df = 5,12)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)


Title:  Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L
File:             4816g            Transform:             NO TRANSFORMATION

                              ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
--------------------------------------------------------------------------------
   Between             5              0.0062           0.0012      94.7185

   Within (Error)     12              0.0002           0.0000
--------------------------------------------------------------------------------
   Total              17              0.0064
--------------------------------------------------------------------------------
                                                          (p-value = 0.0000)

   Critical F  = 5.0643  (alpha = 0.01, df = 5,12)
               = 3.1059  (alpha = 0.05, df = 5,12)
```

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L
File: 4816g Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Neg control	0.0523	0.0523		
2	4.0	0.0541	0.0541	-0.6089	
3	13	0.0514	0.0514	0.2932	
4	41	0.0523	0.0523	-0.0226	
5	128	0.0302	0.0302	7.4653	*
6	421	0.0033	0.0033	16.5555	*

Dunnett critical value = 2.5000 (1 Tailed, alpha = 0.05, df = 5,12)

Title: Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L
File: 4816g Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	4.0	3	0.0074	14.1	-0.0018
3	13	3	0.0074	14.1	0.0009
4	41	3	0.0074	14.1	-0.0001
5	128	3	0.0074	14.1	0.0221
6	421	3	0.0074	14.1	0.0489

Title: Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L
File: 4816g Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	0.0523	0.0523	0.0532
2	4.0	3	0.0541	0.0541	0.0532
3	13	3	0.0514	0.0514	0.0519
4	41	3	0.0523	0.0523	0.0519
5	128	3	0.0302	0.0302	0.0302
6	421	3	0.0033	0.0033	0.0033

Title: Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L
File: 4816g Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	0.0523				
4.0	0.0532	-0.3045		1.7800	k= 1, v=12
13	0.0519	0.1353		1.8700	k= 2, v=12
41	0.0519	0.1353		1.9000	k= 3, v=12
128	0.0302	7.4653	*	1.9200	k= 4, v=12
421	0.0033	16.5555	*	1.9300	k= 5, v=12

s = 0.0036

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	47.	26.	85.	0.12	0.55
EC10	60.	36.	1.0E+02	0.10	0.60
EC25	92.	62.	1.4E+02	0.079	0.68
EC50	1.5E+02	1.1E+02	1.9E+02	0.056	0.76

Slope = 3.34 Std.Err. = 0.531

Goodness of fit: p = 0.98 based on DF= 3.0 12.

4816G : Glufosinate-ammonium & A. flos-aquae 96-hr grwth; ug/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	0.0523	0.0529	-0.000634	100.	0.00
4.00	3.00	0.0541	0.0529	0.00117	100.	9.01e-06
13.0	3.00	0.0514	0.0529	-0.00149	100.	0.0225
41.0	3.00	0.0523	0.0512	0.00116	96.7	3.27
128.	3.00	0.0302	0.0304	-0.000247	57.6	42.4
421.	3.00	0.00333	0.00329	4.14e-05	6.22	93.8